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Effect of Plant Spacing, Nitrogen Fertilisation, Post-Harvest Defoliation and Finger Harrowing in the Control of *Botrytis cinerea* Pers. in Strawberry

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Summary

Four alternatives to chemical control of *Botrytis* in strawberries were assessed, plant spacing, nitrogen fertilisation, post-harvest defoliation and finger harrowing. Increasing plant spacing generally caused reduced *Botrytis* incidence, but only in one of the two cultivars included and not always significantly. Increased application of nitrogen (0 to 80 kg N ha^{-1}) caused increased incidence of *Botrytis* in one of three years of trial and had generally no effect on total yield. Post-harvest defoliation, though having a positive effect on total yield in some instances, only reduced the incidence of *Botrytis* significantly in one of three years, when the general level of infection was high. Finger harrowing, primarily being used in weed control as an alternative to chemical control, had a negative influence on total yield, and no consistent effect on *Botrytis* incidence was recorded.

In all treatments, annual variation in *Botrytis* incidence, and differences among cultivars, were more important than variation among treatments. Thus, it is concluded that the use of less susceptible cultivars may be more important than the use of any of the cultural methods included in this experiment. Optimising cultural methods may, however, be of greater importance in susceptible strawberry cultivars with their greater potential losses due to *Botrytis*.

Zusammenfassung

Der Einfluss von Pflanzabstand, Stickstoffdüngung, Nachernteentblätterung und Striegeln auf den Befall mit *Botrytis cinerea* Pers. bei Erdbeeren. Der Einfluss des Pflanzabstands, der Stickstoffdüngung, der Nachernteentblätterung und des Striegelns wurde bei den Sorten ‘Korona’ und ‘Elsanta’ als jeweilige Alternative zur chemischen Bekämpfung von *Botrytis* bei Erdbeeren untersucht. Weite Pflanzabstände reduzierten den *Botrytis*befall, allerdings nur bei ‘Korona’ und häufig statistisch nicht signifikant. Steigende Stickstoffmengen (0 bis 80 kg N ha^{-1}) erhöhten den Befall in einem von drei Jahren, hatten aber keinen Einfluss auf den Ertrag. Striegeln, das vor allem zur mechanischen Unkrautbekämpfung durchgeführt wird, war ohne Einfluss auf den *Botrytis*befall, reduzierte aber den Ertrag. Nachernteentblätterungen können den Ertrag erhöhen, *Botrytis* wurde aber nur in Jahren mit sehr hohem Befall reduzieren.

Die Unterschiede im *Botrytis*befall zwischen den Jahren und den Sorten waren wesentlich größer als der Einfluss der vier Behandlungen. Deshalb ist der Einsatz wenig empfindlicher Sorten zur Reduzierung des *Botrytis*befalls wesentlich entscheidender als die hier geprüften Behandlungen. Beim Anbau von sehr anfälligen Sorten ist die Optimierung der Kulturtechnik von großer Bedeutung.

Introduction

The Danish environmental authorities have critically reviewed all pesticides used in Danish agriculture and horticulture during the last few years, and a considerable number of pesticides have been banned. Due to this situation, the environmental authorities have sponsored a research project in strawberries with the aim of investigating the effect of cultural methods in the control of grey mould (*Botrytis cinerea* Pers.). The alternatives included were plant spacing, lowering of nitrogen fertilisation, post-harvest defoliation and finger harrowing. The treatments mentioned above are known to affect the occurrence of *Botrytis* to various degrees and recent research results are reviewed in a previous publication (DAUGAARD 1999).

Materials and Methods

A+ frigo plants of two strawberry cultivars (‘Elsanta’ and ‘Korona’) were planted in matted row system in May 1997. Each plot consisted of 6 rows with 15 plants in each row. The plant spacing in the row was generally 0.37 m, except in the experiment concerning plant spacing, where distances of 0.25, 0.37 or 0.50 m were used. The row spacing was 0.90 m and 6 replications were included in all experiments. For practical reasons, the experiment was planted in two parts 100 m apart, one including plant spacing and nitrogen fertilisation treatments, and the other including the post-harvest defoliation and finger harrowing treatments. All flowers were removed by hand six weeks after planting. During the season, the plots were fertilised during flowering

according to soil and leaf analyses and irrigated in dry periods. In the fertilisation treatment, however, three levels of nitrogen were used (0, 40 and 80 kg N per hectare, respectively). The post-harvest defoliation treatment was carried out immediately after harvest, 10 cm above soil level with a tractor driven cutter, and the cut plant material was cultivated into the soil. Weeds were controlled mechanically including the use of rotary cultivator 2–3 times per year and supplementary hand hoeing in the rows. In the plots including finger harrowing, this treatment was added three to four times from spring until the beginning of flowering. All runners between rows were removed mechanically after harvest. Runners in the row were allowed, except in the plant spacing experiment, in which all runners were removed by hand leaving the original plants as separate plants. The plants were covered during the winter period with agryl (synthetic mulch). Pests were controlled according to normal practice, but in order to assess the infestation of *Botrytis* correctly, no fungicides were applied during cultivation. The experiment was extended to three cropping years in order to follow the development of *Botrytis* incidence for as long as possible. Each of the three years, six tonnes of cut wheat straw per hectare was placed between rows after flowering, and after harvest, it was cultivated into the soil. Berry quality, size and yield were recorded for 12 plants situated in the middle of the two central rows of each plot. The remaining plants were considered guard plants. Berries were picked twice a week from all plants, including the guard plants. The total weight of berries, *Botrytis*-infested berries and other not marketable berries was recorded separately.

All data were subject to statistical analysis using the General Linear Model of SAS (SAS Institute, Inc., 1989–95, Cary, NC). The least significant differences between means were determined at $P<0.05$ using Duncan's test.

Results

Climate conditions

The climate in Denmark is mild-temperate and with considerable annual variation. This was also the case during this experiment as illustrated in table 1. Especially during flowering (May) and again during harvest (June–July), differences in weather conditions may result in different levels of *Botrytis* infestation among years. During the first cropping year, January – April was relatively mild and wet, whereas May – June was normal and July – August was colder than normal. During the second year, January – April was considerably milder than normal and rainfall nearly doubled. The average temperature was below normal and with heavy rainfalls in June, whereas July and August were warm and dry. Finally, the third year of trial was characterised by very mild weather from January to May and, especially during flowering in April – May, very warm and dry. Contrary to this, the summer months (June – August) were chilly with precipitation below normal.

Table 1. Monthly climatic records for the experimental site 1998, 1999 and 2000, compared to a 30-year average.

Month	Mean temperature (°C)				Precipitation (mm)			
	1998	1999	2000	1961–90	1998	1999	2000	1961–90
January	2,7	2,4	3,0	–0,2	82	99	48	57
February	5,0	0,6	3,5	–0,4	33	96	63	38
March	3,9	4,0	3,9	1,7	54	73	57	46
April	6,9	8,1	8,7	6,4	86	31	47	41
May	11,8	11,3	12,8	11,3	38	43	31	48
June	14,5	13,5	13,9	14,7	75	102	44	55
July	14,6	17,4	14,8	16,6	76	47	30	66
August	14,9	16,7	15,3	16,3	44	67	45	67
September	13,5	16,5	13,3	13,1	44	35	63	73
October	8,6	9,3	10,9	8,6	178	55	70	76
November	2,0	5,4	6,9	4,9	76	39	59	79
December	1,2	2,4	3,9	2,1	80	117	49	66
Average/Total	8,3	9,0	9,2	7,9	866	804	606	712

Plant spacing

Results concerning the effect of plant spacing on total yield, incidence of *Botrytis* and berry size are outlined for the three years of trial in table 2. Results are shown for both cultivars combined as well as separately. When data for both cultivars combined are considered, the highest total yield was obtained at the lowest plant spacing (25 cm) in all cropping years. Differences were, however, not significant in all cases. Based on a 3-year average and both cultivars, the total yield at a 25-cm plant distance was 28.0 tonnes per hectare declining to 21.2 t ha^{-1} at 50 cm, a difference of 32 %. The two cultivars responded very differently to variable plant spacing as well as plant age. The total yield of 'Korona' was at the highest level the first cropping year and with significant differences between plant distances. The second and third year, the total yield declined, and with less differences between plant distances. As far as 'Elsanta' was concerned, the highest total yield and the most pronounced effects of plant spacing were obtained during the second cropping year. During the first year, the yield of 'Elsanta' was low and with no significant effects of plant spacing and in third year yield was intermediate.

A marked difference among years in *Botrytis* incidence was observed when data for both cultivars were considered. During the first and third cropping year, the level of infection was low (<9 %) whereas the yield loss caused by *Botrytis* was considerable (14–37 %) in the second year. Only in the third year of 'Elsanta', a significant effect of plant spacing on *Botrytis* was found. On average, there was a yield loss of 3.9 t ha^{-1} from 25 to 50-cm plant spacing. The results clearly demonstrated differences in susceptibility among varieties, 'Korona' being a more susceptible cultivar than 'Elsanta'. Especially in the second year of trial, with high incidence of *Botrytis*, the percentage of infected berries in 'Korona' was twice as high as in 'Elsanta'.

Effects on berry size were limited to the third cropping year in 'Korona', showing an increase in berry size with higher plant spacing. Generally, similar tendencies were seen for the other treatments, but the results were not significant.

Table 2. Effects of plant spacing on total yield of strawberries, incidence of *Botrytis* and berry size in 1998, 1999 and 2000.

Cultivar/ Plant spacing	Total yield (tonnes per hectare)			*Botrytis* incidence (% of total yield)			Berry size (grams per fruit)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Both cultivars									
25 cm	27.3 a	35.3 a	21.5 ab	4.6 a	27.5 a	5.8 b	19.9 a	17.2 a	10.8 a
37 cm	23.3 a	32.0 a	23.0 a	5.4 a	26.0 a	8.0 a	19.8 a	17.2 a	11.3 a
50 cm	20.6 a	25.3 b	17.6 b	6.8 a	21.5 a	6.0 ab	19.7 a	17.0 a	12.0 a
'Korona'									
25 cm	37.5 a	32.6 a	17.5 ab	4.5 a	37.1 a	5.3 a	17.2 a	15.9 a	9.2 a
37 cm	32.8 b	33.1 a	18.8 a	6.0 a	34.7 a	7.7 a	18.0 a	15.7 a	9.7 a
50 cm	28.2 c	27.9 b	14.8 b	5.6 a	29.1 a	6.9 a	18.5 a	16.9 a	10.6 b
'Elsanta'									
25 cm	17.1 a	38.0 a	25.6 a	4.5 a	17.8 a	6.3 ab	22.7 a	18.5 a	12.2 a
37 cm	13.8 a	30.8 b	27.3 a	4.7 a	17.2 a	8.2 a	21.6 a	18.6 a	12.8 a
50 cm	13.1 a	22.6 c	2.03 b	7.9 a	13.9 a	5.1 b	21.0 a	17.0 a	13.3 a

Table 3. Effects of nitrogen fertilisation on total yield of strawberries, incidence of *Botrytis* and berry size in 1998, 1999 and 2000.

Cultivar/ N (kg per hectare)	Total yield (tonnes per hectare)			*Botrytis* incidence (% of total yield)			Berry size (grams per fruit)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Both cultivars									
0	26.4 a	31.7 a	24.5 a	2.9 b	23.7 a	5.8 a	18.8 a	14.9 a	11.2 a
40	26.9 a	31.1 a	25.2 a	6.2 a	27.6 a	6.7 a	19.0 a	16.2 a	11.4 a
80	27.5 a	31.0 a	22.6 a	7.5 a	30.1 a	7.3 a	19.8 a	16.6 a	11.7 a
'Korona'									
0	34.1 a	30.7 a	17.7 a	4.2 a	31.8 a	5.0 a	16.9 b	14.8 a	9.8 a
40	35.3 a	31.2 a	17.8 a	7.1 b	34.5 a	7.0 a	17.5 ab	14.9 a	10.0 a
80	36.5 a	28.5 a	19.3 a	10.1 c	34.5 a	7.7 a	18.4 a	14.9 a	10.3 a
'Elsanta'									
0	18.8 a	32.8 ab	31.3 a	1.6 b	15.5 c	6.6 a	20.7 a	15.0 a	12.7 a
40	18.5 a	31.0 b	32.7 a	5.3 a	20.6 b	6.4 a	20.6 a	17.5 ab	12.8 a
80	18.5 a	33.5 a	25.9 b	5.0 a	25.6 a	6.9 a	21.3 a	18.2 b	13.1 a

Nitrogen fertilisation

The effect of nitrogen fertilisation on total yield, *Botrytis* incidence and berry size is recorded in table 3. When data for both cultivars combined are considered, the total yield was unaffected by nitrogen supply levels in all three cropping years. As was the case in the plant spacing trial, the cultivars responded differently with 'Korona' yields not being influenced by the N fertilisation levels used in the trial. 'Elsanta', however, had a negative yield response to increasing amounts of nitrogen added in the last of three cropping years.

The effect of nitrogen fertilisation on *Botrytis* incidence, when data for both cultivars were included, varied between years, as was the case for the plant spacing trial. In addition, the amount of *Botrytis*-infested berries increased significantly with the amount of nitrogen added in the first cropping year for both cultivars, but this effect was only observed during the second year in 'Elsanta'. During the third year, no effect of nitrogen fertilisation level on *Botrytis* incidence was found. Generally, *Botrytis* incidence was much higher in 'Korona' than in 'Elsanta', and, accordingly, differences between nitrogen levels. The second year of cropping, with high levels of *Botrytis* infection, 'Korona' was heavily attacked regardless of nitrogen level, whereas in 'Elsanta' there were still significant differences between nitrogen levels.

In general, the berry size declined from the first through to the third year of harvest. There was, however, a significant increase caused by increasing amounts of nitrogen added, for 'Korona' the first cropping year and for 'Elsanta' the second cropping year. Although not significant, the other years had the same tenden-

Table 4. Effects of post-harvest defoliation or finger harrowing on total yield of strawberries, incidence of *Botrytis* and berry size in 1998, 1999 and 2000.

Cultivar/ Treatment	Total yield (tonnes per hectare)			*Botrytis* incidence (% of total yield)			Berry size (grams per fruit)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Both cultivars									
Defoliation	25.1 a	34.3 a	25.6 a	3.2 a	19.3 a	6.2 a	18.3 a	18.6 a	15.8 a
Finger harrowing	16.8 b	32.3 a	19.4 b	2.0 ab	26.0 a	11.6 a	16.9 a	17.9 a	14.5 ab
Control	21.0 ab	30.1 a	24.7 a	1.5 b	29.2 a	8.8 a	18.5 a	17.1 a	13.8 b
'Korona'									
Defoliation	31.5 a	40.6 a	28.2 a	4.3 a	23.5 c	9.4 b	16.9 a	18.1 a	14.6 a
Finger harrowing	21.9 b	32.0 b	20.8 b	2.6 b	35.9 b	17.4 a	15.8 a	16.1 a	12.8 b
Control	28.5 a	26.1 c	27.3 a	2.1 b	45.4 a	11.1 ab	17.2 a	15.6 a	12.8 b
'Elsanta'									
Defoliation	18.8 a	27.9 b	23.5 a	2.1 a	15.1 a	3.5 a	19.7 a	19.1 a	16.7 a
Finger harrowing	11.7 b	32.7 a	17.9 b	1.5 a	16.2 a	5.8 a	18.0 b	19.8 a	16.1 a
Control	13.6 b	34.1 a	21.4 ab	1.0 a	15.6 a	5.9 a	19.8 a	18.6 a	15.0 a

cies. Apparently, with increasing amounts of nitrogen added, the plants produced fewer, but bigger berries.

Post-harvest defoliation

The effects of defoliation on total yield, *Botrytis* incidence and berry size are recorded in table 4. The combined yield data for both cultivars showed increases in total yield following defoliation, although this was only significant in one of three cropping years. As shown in the individual data for each cultivar, the positive yield response to post-harvest defoliation varied with cultivar and cropping year. In fact, the second cropping year there was even a negative yield response to defoliation in 'Elsanta'.

While post-harvest defoliation showed some yield effects in both 'Korona' and 'Elsanta', a positive response on *Botrytis* incidence was generally limited. Positive effects on *Botrytis* incidence were only seen in 'Korona', where the percentage of *Botrytis*-infested berries was significantly reduced in the second and third cropping year. In 'Elsanta', being less susceptible to *Botrytis* in general, no effects of defoliation were evident.

Post-harvest defoliation caused a slight increase in berry size, although significant only during the third cropping year.

Finger harrowing

The effects of finger harrowing on total yield, incidence of *Botrytis* rot and berry size are also shown in table 4. The combined data for both cultivars indicate a reduction in total yield following finger harrowing compared with a control. The negative effect of finger harrowing was significant in 'Korona', whereas 'Elsanta', apparently, was less sensitive to this treatment. The reduction in yield of 'Korona' was 13 % on average. Being a relatively vigorous cultivar (DAUGAARD 1999) it seems probable that finger harrowing damaged the leaves of 'Korona' resulting in yield reduction.

Contrary to the yield effects of finger harrowing, no consistent effects on *Botrytis* incidence was recorded. A reduction in *Botrytis* infection was observed only in 'Korona' and only during the second cropping year, when the level of infection was high.

Discussion

Plant spacing

As relative humidity is an important factor in the biology of *Botrytis*, good aeration around strawberry plants is expected to influence disease incidence. A number of research results have shown good correlation between *Botrytis* incidence and plant spacing (DIJKSTRA and VAN OOSTEN 1985; SUTTON et al. 1988; SØNSTEBY et al. 1996; LEGARD et al. 2000). Some investigations, however, report little or negligible effects (WILCOX et al. 1994; MEÅS and SOLBERG 1996). Plant spacing in this connection is defined as distance between plants as well as row distance. Most of the experiments on this topic, however, were carried out according to standard disease control practices using chemical fungicides, which may reduce the effect of plant spacing on *Botrytis* incidence. In organic farming, where no synthetic pesticides are used, a single row system is generally recommended to secure good aeration around the strawberry plants (MARTINSSON 1988; DAUGAARD 1996; SCHMID 1996). According to DIJKSTRA and VAN OOSTEN 1985 and STRIK et al. 1997, double or triple row systems cause increased incidence of *Botrytis*, which makes these systems unsuitable in organic as well as IPM systems. In our experiment, there were marked differences between the cultivars in *Botrytis* incidence, indicating that cultivar resistance may be more important in the control of *Botrytis* than plant spacing. Finally, effects of plant spacing on *Botrytis* incidence was only important in one of three years when the general level of infection was very high, with more than 25 % of the total yield destroyed by *Botrytis*. In years of low level of infection, no effect of plant spacing was recorded. The good correlation between yield and plant spacing confirmed previous experiments (BRANDSTVEIST 1979; WILSON and DIXON 1988).

Nitrogen fertilisation

Strawberry plants in general require only small amounts of nitrogen (DAUGAARD 1987). Although in one case yield was positively correlated to nitrogen supply up to 80 kg N ha^{-1} (KOLBE 1977), in most cases high

nitrogen supply (up to 150 kg N ha^{-1}) did not cause increased yields (BRANDTSTVEIT 1979; KUNKEL 1987; KOPANSKI and KAWECKI 1994; SØNSTEBY et al. 1996). This was further confirmed in our experiment. Depending on soil type, high amounts of nitrogen cause increased disease incidence and are not recommended (DAUGAARD 1987). The incidence generally increases with increasing nitrogen supply (KOPANSKI and KAWECKI 1994; WILCOX et al. 1994; COOLEY et al. 1996). This was also the case in our experiment in some years. Furthermore, there were significant differences in cultivar responses to nitrogen application, depending on their general susceptibility to *Botrytis*. Variations in the occurrence of *Botrytis* may thus be explained by differences in climatic factors and cultivars as well as nitrogen supply and the occurrence of nitrogen in the soil. In any case, it must be concluded that low nitrogen supply is an important factor in the cultural management of *Botrytis*.

Post-harvest defoliation

As dead leaves are considered the most important inoculum source of *Botrytis* (BRAUN and SUTTON 1987), an obvious strategy to reduce initial inoculum in strawberry fields is to remove or destroy dead plant tissue or, at suitable times, the entire living and dead foliage of the crop (SUTTON 1991). A possible method is removal of the leaf canopy after harvest. Though this is a method, which previously has been practised by many growers, its effect on the incidence of *Botrytis* is uncertain. In one instance it was found that defoliation had a small but significant effect on the incidence of *Botrytis* the year following treatment (NESTBY 1985). In our experiment, post-harvest defoliation caused a significant reduction in *Botrytis* incidence in 'Korona' in the year with a heavy *Botrytis* infestation (1999), but not in 'Elsanta'. On the other hand, several investigations have shown no significant effect of leaf removal on the incidence of *Botrytis* (THORSRUD 1983; WILCOX et al. 1994; COOLEY et al. 1996; LEGARD et al. 1997; DAUGAARD 2000). In the experiment by LEGARD et al. 1997 where conventional fungicide treatment was compared with sanitation (removal of senescent and diseased berries and leaves) and a combination of sanitation and fungicide treatment, sanitation alone did not show any positive effect. When LEGARD et al. 1977 compared spray only and combined spray-sanitation treatments, no significant difference was observed. Furthermore, leaf removal in some cases caused lower yields the following year (SÄKÖ 1975; KUNKEL 1987). The apparently contradictory results may be explained by differences in climate, length of season, choice of cultivars and time and extent of defoliation. In conclusion, removal of dead plant material to reduce the incidence of *Botrytis* is not in itself sufficient, but must be supplemented by other methods.

Finger harrowing

The use of finger harrowing originally is a weed control method, which has recently become more popular in strawberries (JENSEN 1998). Apart from its effect on weed control, it is claimed to cause a reduction of the incidence of *Botrytis* during the removal of dead leaf material (OPSTAD et al. 1998). According to the results of this trial, the use of finger harrowing may cause a reduction in *Botrytis* incidence at least in 'Korona'. In a similar experiment performed previously at our Institute, neither 'Korona' nor 'Senga Sengana' showed any reduction in *Botrytis* incidence following finger harrowing (DAUGAARD 2000). Clearly, there is a need for further experiments regarding the effect of finger harrowing on *Botrytis* incidence before such discrepancies will be clarified.

Conclusion

In strawberries, *Botrytis* is considered the most important fungal disease and no resistance genes are known to exist. However, there is a marked difference among cultivars in susceptibility (DAUGAARD 1999), which was confirmed in our experiments. Furthermore, our experiments clearly illustrated the problems of finding alternative cultural methods to the use of fungicides, as none of the methods assessed gave efficient *Botrytis* control. Although increased plant spacing, reduced nitrogen fertilisation, post-harvest defoliation and finger harrowing in some instances may have positive effects on *Botrytis* infestation in strawberries, the cultivars responded very differently to such treatments. 'Korona', being a very vigorous and susceptible cultivar, responded positively to most treatments, whereas 'Elsanta', which is less vigorous and more resistant to *Botrytis*, responded very moderately or not at all. It may be concluded that the use of less susceptible cultivars is a more important factor than the use any of the cultural methods included in these experiments for controlling *Botrytis*. On the other hand, the use of cultural methods may be of greater importance in susceptible cultivars with their greater potential losses due to *Botrytis*.

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